Monitoring the Anthropogenic Toxicity of Spontaneous Flora in Neamt County through Studies of the Honey Bee Chemical Characteristics

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The products of honey bees can be used as indicators and monitors of a variety of environmental pollutants because of the bees' ability to collect materials that reflect their immediate environmental conditions. Human activities produce more waste and administrate the pesticides, the amounts and toxicity of which often exceed the environment's homeostatic capacity to cleanse itself, and this is constantly transforming due to intensive agricultural practices necessary to increase food production as human population grows. The main sources for contamination of honey with heavy metals are represented by placing hives near urban areas with heavy car traffic, or industrialized areas and the use on the entire circuit of production, objects or containers made of materials unsuitable (unacceptable). For that reason regular monitoring of the environment is so important. Honey bees, thanks to their morphological features, and also bee products are regarded as good indicators of environmental pollution by toxic substances, be these heavy metals, radioactive elements or persistent organic pollutants such as pesticides. Consequently, it is important to estimate the environmental fate and Eco toxicological effects of these different xenobiotic. Honey bees (Apis mellifera L.) have been used as biological indicators of Plant Protection Products (PPPs) in two intensely cultivated in areas of Neamt County, Romania. This area is representative for the pre-mountain and mountain zone of Romania. The stratified sample has been face-to-face interviewed in 2016 regarding the data from the year 2015. The total consumption specific for the honey production was about 628 MJ hive^{-1}, and the energy output reached 235 MJ hive^{-1}, determining an energy productivity of 0.030 kg MJ^{-1} and an energy use efficiency of 0.37. Specific energy amounted 33.3MJ kg^{-1} due to the inefficiency of traveling during the apiaries movements and the inappropriate correlation between the apiaries size and the zonal melliferous potential. In this paper available literature data and information on the morphological features of the honey bee, the utilization of the honey bee and its products as indicators of environmental pollution, and a historical outline of some of the legislation relating to beekeeping have been critically compared and discussed.

Keywords: environment, indicators, monitors, energy efficiency, beekeeping management, honey production

This study was conducted with an aim to identify the existing honey bee production practice, constraints and potentials and to suggest possible intervention measures for future improvements. Honey bees are considered good biological indicators because they reveal the level of habitat degradation. Disclosure is transmitted by two signals: one is more obvious and is expressed by high mortality (in the case of pesticides), while the latter is less obvious (in case of heavy metal contamination) and is expressed by accumulation of residues in their bodies, diminishing productivity and affecting the harmlessness of apiculture products, both signals can be detected by means of the respective laboratory analyzes [1].

Honey is the sweetest product of the nature, made by processing the nectar of flowers or plants blight. The chemical composition of honey consists of: water, glucose, fructose, sucrose, dextrin, vitamins of group B, C, provitamin A, P and K, pantothenic acid, folic mineral salts of Ca, Na, P, Al, Fe, Si and Mg and some trace minerals: Ni, Ag, V and Cr [2].

Beekeeping monitoring could present an important element in the range of measures to assess the impact of pollutants in an ecosystem. Bees, by absorbing pollutants directly from air or water, become indirectly through pollen and nectar a possible source of pollution of bee products [3].

The contents of trace elements were determined by atomic absorption spectrometry after application of microwave digestion. The samples from the thermal power plants, which were 10–22 km away from the hives, that did not cause pollution in honeydew honeys were also analyzed. The levels of copper, cadmium (Cd), lead (Pb), zinc, manganese, iron, chromium, nickel, and aluminum were similar to the values found in other recent studies in literature. However, it was found that the contamination levels of the toxic elements such as Pb and Cd in honeybee samples measured relatively higher than that of honey samples. The study concludes that honeybees may be better bioindicators of heavy metal pollution than honey.

The pesticide pollution in intensively cultivated areas represents a dangerous phenomenon because these products accumulate in vegetation, water and soil and cause damages to beneficial organisms such as honey bees (Apis mellifera L.) [4]. Honey bees commonly forage within 1.5 km of their hive and exceptionally as far as 10 to 12 km, depending on their need for food and its availability [3]. The honeybee is a good biological indicator that quickly reflects chemical impairment of the environment by its high mortality and the presence of pollutants in its body or in beehive products. In this work the honeybee (Apis mellifera) and honey were used to detect the presence of polycyclic aromatic hydrocarbons (PAHs) in several areas

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with different degrees of environmental pollution. All sampling sites showed the presence of PAHs. Benzo(a) pyrene was never detected. Fluorene, phenanthrene, anthracene, fluoranthene, benz(a)anthracene, benzo(b) fluoranthene, and benzo(k)fluoranthene were the PAHs detected in bees, whereas the honey contained only phenanthrene, anthracene, and chryse.

Many studies have demonstrated that honey bees can be used successfully to sample an area for environmental contaminants [5].

The apiculture is fully acknowledged for the contribution brought to the ensurance of human wellbeing and environment support [6].

On the contrary, this branch is facing a series of severe problems as illnesses, pests, pollution, pesticides, crop rotations with a reduced number of cultures etc, and especially those with deficit management [7].

Apiculture is also considered an important source of positive externalities through the pollination of spontaneous and cultivated entomophily plants [8] but the net energy is usually negative.

The highest energy inputs were those related to the transport and fuel, representing 80.3% out of the total inputs. The indirect inputs reached 768.5 MJ·hive^{-1}. The non-renewable energy inputs represents 96.5% out of the consumed total energy, value that may be improved by preventing the bees swarming, nest organization for wintering, nest resizing in the active season and the families preparation for the production harvest.

In many countries of the world, more and more frequently, emphasis is placed on the concept of environmental monitoring through the honeybee (Apis mellifera L.) or the newer apimonitoring [9-10].

Experimental part

Materials and methods

The research from the topic announced was conducted in research laboratory of the Agrochemistry Department from the Faculty of Agriculture (USAVM-Iasi). The purpose of the paper is to observe specific variations in the content of heavy metals of honey, following differentiated location of the hives. We consider 2015 the year when hives were placed in areas with differentiated impact of pollution, making it possible to identify the influence of the pollution sources mentioned on the quality of honey, by identifying residues of heavy metals.

The sampled named P1 are close to the polluting source (Mixed Flowers P_1 and Linden Tree honey P_1). The hives from the areas mentioned above were kept in the same position during this study. The samples of honey were examined in the same conditions and using the same procedures.

The sample analysis has followed standard methods for detecting heavy metals approved by STAS 784/2-2009, in Romania. Atomic absorption spectrophotometry (AAS) was used for heavy metal detection.

The research was carried out during the period 2015-2017. In the first year of research (2015) samples of apiculture products (honey) were collected from several localities of Neamt county.

The level of environmental pollution can be determined by physical, chemical and bio indicator methods. More recently, particular attention is given to living organisms as indicators of environmental health. Bio indicators of pollution may be animal (honey bees, ants, etc.) and vegetal ones, the latter being more numerous.

Sampling flowers

Of the 4 experimental sites (25 May - 5 June of each year), 12 samples of honey flowers were collected at different distances from the experimental steppe within 3 km. The flowers were packed in plastic bags and transported to the laboratory for analysis.

Sampling of bee and apiculture products (pollen, honey and propolis) was performed with sterile instruments. In the first year of research, honey samples were taken from 10 districts of the republic (north, center, south) and in the next two years, 12 samples of bees were taken from each study site (from the experimental hives) pollen, honey and propolis.

Was used 50 work bees were taken and placed in containers with a drilled lid. For pollen collection, pollen collectors were installed in front of each urn. After 5 hours of picking, the pollen from the collectors was transferred to containers with a bee-keeping brush.

Honey samples were taken (after 2 weeks) by cutting with a scalpel of a honeycomb portion (10 x 10 cm) and stored in containers.

Propolis samples were taken using the beehive chisel and stored also in containers.

The samples mentioned above were transported to the laboratory for analysis. In order to achieve the proposed objectives, 162 samples of environmental components and 84 samples of bees, pollen, propolis and 94 samples of honey were analyzed during the years 2015-2017. The analysis of the researches was carried out in Geolab IGS laboratory of the ASM, and in the chemistry laboratory of USAMV-Iasi. In this way I would like to thank those who have contributed to the laboratory tests.

The analysis of heavy metals (Pb, Cd and Cu) in the environment components (soil, water and flowers) and apiculture products (pollen, honey and propolis) was performed by flame atomization and electro thermal atomic absorption spectrometry [11]. The method is widely used both for the determination of heavy metals in the environment and in apiculture products.

To determine the amplitude of the concentrations and assess the degree of chemical pollution of soil, water, flora, bees, pollen, honey, heavy metals (Pb, Cd and Cu) propolis the concentration of the total forms was determined. For the detection of heavy metals, the AAnalist 800 Spectrophotometer with Perkin Elmer lamps was used to quantify Pb, Cd and Cu.

After carbonization, the samples were gradually mineralized into the electric furnace, increasing the temperature by 50°C every 30 min up to 450°C. Mineralization was continued at this temperature until gray ash was obtained.

The ash beaker, removed from the electric furnace after 10-15 hours of calcination, was cooled to room temperature and moistened with 0.5-1.0 cm^3 of the nitric acid solution.

The acid was evaporated to dryness on the electric wire at low heating. After cooling, the sample vessel was again placed in the cooled electric furnace. Gradually the temperature was increased to 300°C, and then held for 30 min. This cycle was repeated at least twice. Mineralization was considered complete when the ash became white or almost white without carbonized particles.

Analyse of the ash solution. For the determination of heavy metals from ash solutions, the atomic absorption atomic absorption spectrometry (GFAAS) method was used in the atomic absorption spectrophotometer [12].

Performing the analysis. Samples were placed in the oven cup successively, according to a predetermined schedule. The volume of solution injected was 20 μL. Inert gas served argon. The measurement of the atomic absorbance of each sample was performed twice. Compensation of non-selective absorption was achieved with Zeeman corrector [13].

The research was focused on the apiculture in Neamt County because this area is representative for the hill and mountain zone of Romania.

Neamt County is situated in the Eastern-Central part of Romania, is crossed by the meridians 260 and 270 Eastern longitude and the parallel 470 Northern latitude, it has an area of about 5,890 km^2 (2.5% from the Romania's surface) and occupies a part from Bistrita, Moldova and Siret rivers basins.

The mountain and hill zone included into the researched sample represents 69% of the county area. The climate is continental, with particularities specific to the Eastern part of the country. The absolute maximum temperature is about +38.6°C (1952), while the minimum temperature reaches -33.2°C (1954) (http://www.neamt.insse.ro).

The obtained data have been processed by specific IT applications (SPSS, MS Office, ANOVA), the data testing used Kolmogorov–Smirnov, t-test, data analysis: structure, correlation, linear regression [12]. The used indicators have been the following [8]:

$$\text{Net energy } (MJ \cdot have^{-1}) = \text{energy output } (MJ \cdot have^{-1}) - \text{energy input } (MJ \cdot have^{-1}) \quad (1)$$

$$\text{Energy use efficiency} = \text{energy output } (MJ \cdot have^{-1})/\text{energy input } (MJ \cdot have^{-1}) \quad (2)$$

$$\text{Energy productivity } (kg \cdot MJ^{-1}) = \text{product output } (kg/have)/\text{energy input } (MJ \cdot have^{-1}) \quad (3)$$

$$\text{Specific energy } (MJ \cdot kg^{-1}) = \text{energy input } (MJ/have)/\text{product output } (kg/have) \quad (4)$$

The energy inputs were calculated by multiplying the consumed quantities with the specific energy supply: Human labor - 1.96 MJ h^{-1} [12], fuel - 56.31 MJ/L, electricity - 11.93 MJ/kWh, track - 10.15 MJ·km/t, drugs - 13.64 MJ/kg, sugar - 15.4 MJ/kg. The energy outputs were calculated as the product between the honey quality multiplied with the energy supply specific for honey - 12.72 MJ/ kg [14].

The indirect energy inputs included track and diesel fuel because they participate to the transport of honey, hives, raw materials and materials and are not implicated directed into the honey production. The fuel is used by the beekeepers having their own automobiles. The direct inputs are human labor, drugs, electricity and sugar [14]. As renewable input is the labor, and as non-renewable inputs are all the other categories [15].

The stratified sample applied (0-50, 51-100, 101-150, over 151) included 77 beekeepers and it was accomplished by Neyman method, with a deviation criteria of 5% and a trust level of 95%. The field research was carried out through face-to-face interview, at the subject's home, with the apiaries' owner or administrator, during the periods February -April and October-December 2016.

The beekeepers had been questions regarding the obtained productions, the consumptions accomplished during the year 2015 and the applied technology.

Results and discussions

The 42,864 beekeepers of Romania own 1.39 million bee families, they got a honey production of about 27,893 tones/year in 2015, with an average of about 23,960 tones/ year (2011-2015). The average number of bee families owned by a beekeeper amounted 34.4, while the average honey production reached 558 kg/apiary and 17.2 kg/hive [16]. The honey selling is carried on directly to the clients, on the agri-markets, within the Association of Beekeepers and to the exporters.

The total distance of the apiaries movements varies from 50 km to over 1,000 km. The distance between the hives in the wintering courts is about 40-70 centimeters during the pastoral time and about 10-50 centimeters between the hives from the bee pavilions, respectively for the hives placed on soil.

The bee's attractiveness as an ecological detector is based on several peculiarities of morphological and ethological characters, such as: high breeding rate of bee families, low life span, facilitating the holding of pollutant particles on her body due to bark cover, increased sensitivity to toxic substances, high mobility, allowing monitoring of an enormous area of the environment [17, 18].

Determination of the content of heavy metals (Pb, Cd and Cu) in the components of the environment (soil, water and flowers) and apicultural components (bees, pollen, honey and propolis); determination of pesticide residues (organophosphorus and pyrethroids) in environmental compartments and apicultural components; elucidating the correlation of environmental pollution with pollutants in the honey bee body and its products; research into the influence of pollutants on the viability and productivity of bee families; development of proposals for environmental quality assessment.

The work is intended monitoring environmental quality through honeybee regarding the content and distribution of heavy metals (Pb, Cd and Cu) and pesticides (organochlorine, organophosphate and pyrethroid) in the environmental components (soil, water and flowers), bees and bee products (pollen, honey and propolis) in areas with different anthropic impact. It was studied the influence of heavy metals on the bees families viability and productivity. It was elucidated the relationship of heavy metals and pesticides concentration in the environmental components and their concentration in the bees body and bee products.

Natural honey does not have or contains very small amounts of hydroxymethylfurfural (HMF), the standard in force limiting this indicator to a maximum of 1.0 mg/100 g and to the one packed in jars at 4.0 mg/100 g.

Therefore, it is considered that a high quality bee honey contains not more than 1.5 mg/100 g of hydroxyl-methylfurfural, the acceptable quality is not more than 4.0 mg/100 g and the heat treated honey crystals) contains not more than 100 mg/100 g (table 1).

Currently in the EU it is estimated that 4.0 mg/100 g is a barrier too restrictive, especially for products in the subtropical area, and it is considered that a content of up to 8.0 mg/100 g is acceptable for this category of products. To identify hydroxy-methylfurfural, qualitative (Fiehe) or quantitative (Winkler) methods can be used.

Neamt County showed the presence of metal residual levels in all samples analyzed. Residual levels of metals found in honey samples are reported in table 2.

As seen in the table above, in the samples analyzed was not detected: Cd, Cr and Ni were detected only in Linden Tree honey [19].

Honey contains many mineral substances, the quantity of which varies very widely, being valued at a maximum of 0.35% for floral honey (most of the time however being around 0.1%), while honey has a much richer content that may be close to 1%.

The most well represented elements are potassium (which represents almost half of the mineral substances), chlorine, sulfur, calcium, sodium, phosphorus, magnesium, silicon, iron, manganese and copper, the content of which is presented in the table below (table 3).

Parameter	Honey of Acacia*		Honey of manna		The other sorts
	Cal. sup	Cal. I	Cal. sup	Cal. I	Cal. I
Water, % max.	20	20	20	20	20
Acidity, 1 N NaOH/100g max.	4	4	5	5	4
Reducing sugar, expressed as invert sugar, %, min.	70	70	60	60	70
Slightly hydrolyzable sugar expressed as sucrose, %, max	5	5	10	10	5
Amylase index, min.	6.5	6.5	13.9	10.9	10.9
Hydroxymethylfurfural, mg/100 g max.	1.5	1.5	1.5	1.5	1.5**)
Ash, %, max.	0.5	0.5	1	1	0.5

** In addition, high quality acacia honey must not contain more than 5% rape seed pollen and/or fruit trees;*

*** Honey delivered in jars is permitted to contain an HMF content of max. 4 mg per 100 g.*

Table 1
WASTE LANDFILLED AND INCINERATED

Sample	Cr	Ni	Zn	Cu	Mn	Cd	Fe
Mixed Flowers P_1	-	-	0.987	18.89	0.512	-	10.49
Mixed Flowers P_0	-	-	-	39.55	0.044	-	17
Linden P_1	10.34	7.64	0.992	35.543	2.526	-	80.38
Linden P_0	6.875	0.664	0.1477	75.5	4.803	-	67.89
Rape	-	-	0.336	48.17	1.284	-	47.24
Acacia	-	-	1.48	10.73	6.31	-	23.18

Table 2
HEAVY METALS RESIDUES IN HONEY (ppm) [19]

Elements	Light honey			Honey pronounced color		
	Average	Minimum	Maximum	Average	Minimum	Maximum
Potassium	205	100	588	1.676	115	4.733
Chlorine	52	23	75	113	48	201
Sulfur	58	36	108	100	56	126
Calcium	49	23	68	51	5	266
Sodium	18	6	35	76	9	400
Phosphorus	35	23	50	47	27	58
Magnesium	19	11	56	35	7	126
Silicon	8.9	7.2	11.7	14	5.4	28.3
Iron	2.4	1.2	4.8	9.4	0.7	35.5
Manganese	0.3	0.17	0.44	4.09	0.52	9.53
Copper	0.29	0.14	0.7	0.56	0.35	1.04

Table 3
HONEY MINERAL ELEMENTS (mg/kg) [20]

Honey is relatively poor in vitamins compared to other foods and especially to fruits. Contains vitamin C and some B vitamins (thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine and folic acid) but does not contain lip soluble vitamins A, D and E (table 4).

Consequently, the efficient management of resources represents an important objective for the beekeepers aiming to increase the economic benefits, and the energy efficiency meets the current needs of the human civilization, under the conditions of increasing in food necessary and with the hope to increase the humans' wellbeing [21-25].

The research illustrated that the deficit apicultural management consists in the inefficient use of resources as far as the quantity, combination manner, time of use and place of accomplishment are concerned. The toxicity and biochemical changes in honey bees *(Apis mellifera)* treated with four insecticides acetamiprid, dinotefuran, pymetrozine, and pyridalyl-were evaluated under controlled laboratory conditions.

Amino acids	Average concentration (nmoles/g of honey)					
	1	2	3	4	5	6
Lysine	123	170	122	78	107	133
Aspartic acid	90	167	170	124	146	136
Threonine	63	86	110	42	60	92
Serine	70	120	112	62	90	100
Glutamic acid	144	335	298	126	220	246
Proline	2	4.09	3.25	1.870	230	3.14
Glycine	44	76	100	46	66	67
Alanine	73	150	132	70	114	118
Valine	46	76	65	33	82	66
Zsoleucine	43	107	62	30	70	67
Leucine	40	92	80	33	58	60
Tyrosine	55	238	100	120	75	120
Phenylalanine	170	312	180	110	218	208
Sample Number	44	29	17	8	8	191

Table 4
AVERAGE CONCENTRATION OF THIRTEEN FREE AMINO ACIDS FROM SOURCES-FLORAL [26]

Chemical element	Mixed	Rape	Linden tree	Acacia tree	Dark
Pb	0.07	0.03	0.09	0.089	0.18
Cd	0.015	0.008	0.018	0.017	0.027
MAL Pb	0.2	0.2	0.2	0.2	0.2
MAL Cd	0.02	0.02	0.02	0.02	0.02

Table 5
Pb AND Cd CONTENT IN HONEY SAMPLES FROM THE UNPOLLUTED AREAS

Chemical element	Mixe	Rape	Linde	Acaci	Dark
Zn	2.3	0.2	2.6	2.5	3.3
MAL Zn	3	3	3	3	3

Table 6
Zn CONTENT IN HONEY SAMPLES FROM THE UNPOLLUTED AREAS

Foraging bees were exposed to different dosages of tested insecticides by oral feeding at different dosages recommended by the manufacturers for agricultural crops in Romania (0.01-, 0.02-, 0.04-, 0.1-, and one fold). Moreover, the acute toxicity of these insecticides was evaluated by topical application on the thorax of foragers to calculate the LD_{50} values.

The specific activities of acetylcholinesterase (AChE), carboxylesterase, glutathione S- transferase (GST), and polyphenol oxidase (PPO) were measured in different tissues of surviving foragers after 24 h of treatment to explore the possible mode of action of insecticides and honey bees' strategies for detoxification and tolerance. In the tables below the results of the samples from the Apiculture Association of Neamt pollution free areas are presented (table 5).

Also the maximum admitted levels (MAL) of Pb, Cd, and Zn set by OMS 975/88 are shown for comparison (table 6).

As seen in the figures above, the levels of Pb, Cd and Zn are below the (MAL) for all tested honey samples except Dark honey which has a slightly higher level of Zn and Cd than the (MAL).

Another result is that Linden Tree honey and Acacia Tree honey contain higher levels of microelements than Rape honey. This might be due to the fact that perennial plants store heavy metals differently in comparison with trees.

The presence of Zn in the biota is beneficial, being one of its micronutrients, as long as it does not exceed (MAL). The presence of Cd and Pb in honey is already an evidence of micro polluting agents in the environment.

Higher content of Cd and Zn in Dark honey than the (MAL) could be used to assess the qualitative changes induced by pollution to honey [1].

The amount of heavy metals exceeds the (MAL) in Mixed Flowers honey and in Acacia Tree honey coming from the hives situated in Neamt county (closest to the polluting source). Even though in the analyzed samples from the other research areas, the content of heavy metals is below the (MAL), the presence of these micro polluting agents proves that the effect of the polluting clouds.

The content of Pb in the flowers of the honey plants. After laboratory tests, in 2015, we found the presence of Pb and Cd in the collected flowers from all analyzed samples (tables 7 and 8).

Comparing the average Pb content in the honey-flows of the study sites, we found that the lowest Pb concentrations in the flowers were recorded in the *forest* site (0.117±0.025 mg/kg), and the highest concentrations were found in Urban proximity site, with values of 0.252±0.029 mg/kg, being greater than the control site with 0.13 mg/kg or 2.1 times (td = 3.42, p < 0.001). In the *rural* site, Pb concentrations were determined in flowers with average values of 0.252±0.029 mg/kg, being higher than the *forest* site by 0.11 mg/kg or 94.8% (td = 2,40; p < 0.05). Analyzing the Pb concentrations in the flowers in each separate sample, we found that minimum levels of 0.029 mg/kg were recorded in the *forest* site and maximum 0.402 mg/kg in the *rural* site. In 2017, Pb concentrations in flowers showed similar slightly lower levels in the *forest* site (0.141±0.025 mg/kg) and somewhat higher in the agricultural site (0.178±0.018) and the rural site 0.197±0.034 mg/kg). The limits of the Pb concentrations in the flowers in each sample vary between the minimum level of 0.027 mg/kg in the *forest* site and the maximum of 0.461 mg/kg in the *rural* site. Higher Pb concentrations in *urban* and *rural* sites show that the flora in these sites is more exposed to pollution with this metal through soil,

Name of the experimental site	Average concentration, M±m	The difference from the blank site		t_d	p
		d	%		
Year 2015					
The "forest" site	0.117±0.025	-	-	-	-
The "agricultural" site	0.211±0.020	0.09	80	2.90	< 0.05
The "rural" site	0.228±0,039	0.11	94.8	2.40	< 0.05
The site "Urban approaches"	0.252±0.029	0.13	115.4	3.42	< 0.001
Year 2017					
The "forest" site	0.141±0.025	-	-	-	-
The "agricultural" site	0.178±0.018	0.04	26.2	1.23	> 0.1
The "rural" site	0.197±0.034	0.05	39.7	1.19	> 0.1
LMA, UE*	0.30	-	-	-	-

Remark: * Regulation (EC) No. 1881/2006 [13].

Table 7
Pb CONTENT IN HONEY PLANTS (mg/kg)

Name of the experimental site	Average concentration, M±m	The difference from the blank site		td	p
		d	%		
Year 2015					
The "forest" site	0.021±0.005	-	-	-	-
The "agricultural" site	0.029±0.005	0.008	38.09	1.14	> 0.1
The "rural" site	0.040±0.014	0.019	90.4	1.36	> 0.1
The site "Urban approaches"	0.057±0.011	0.036	176.1	3.09	< 0.001
Year 2017					
The "forest" site	0.019±0.004	-	-	-	-
The "agricultural" site	0.022±0.006	0.003	15.8	0.42	> 0.1
The "rural" site	0.031±0.005	0.012	63.2	1.89	< 0.1
LMA, UE*	0.2	-	-	-	-

Table 8
Cd CONTENT IN MELLIFEROUS PLANT FLOWERS (mg/kg)

water and air. This is largely due to exhaust fumes eliminated by car transport, being the main source of pollution in these areas. Overall, in all sites investigated, the concentration of Pb in the flowers did not exceed the LMA established by the rules in force.

The content of heavy metals in honey

Honey is the natural sweet substance produced by honey bees in plant nectar or plant parts secretions or excretions of insects, plant sap, which bees collect and transform them by combining with their own special substances, then store, dehydrate, store them and honeycombs to mature [26].

To produce 0.5 kg of honey, the honeybee must visit 3-4 million flowers and collect and transport 75,000 nectar loads to their colony. By incorporating nectar, carbohydrates, proteins, albuminous substances, acids, minerals, including metals, biologically active substances of organic origin, vitamins, natural antibiotics, hormones and pollen are incorporated into honey. But with these, a number of contaminants such as heavy metals, pesticides, etc. can penetrate. In general, according to research findings [27-31], the presence of metals in honey depends on the botanical origins of honey, the type of soil and the anthropogenic activities taking place in the given site of the given environment.

Pb content in honey

Using Apis mellifera and some of its products as bio indicators in 2015, the Pb content was previously determined in the samples of honey taken from different in Neamt region, in order to elucidate the general situation of the anthropic impact in the country as a whole (table 9).

Research results (2017) have shown that the Pb content in honey samples analyzed in different areas of the Republic ranges from 0.028 to 0.134 mg/kg. Significantly higher concentrations were recorded in the honey collected in the suburbs of the Brusturi, Poiana and Tarzia communes, respectively, being 0.134±0.010; 0.122±0.018; 0.081±0.010 mg/kg.This is explained by the fact that the suburbs of the communes have a higher anthropic impotence. Several heavy metals emitters are present in these areas, such as the cement and metallurgical plant located within the productive flight (Poiana), the fumigation gases from the industry and the car transport coming from the city, and of course the cross-border pollution.

Therefore, taking honey as a bio indicator of the level of environmental pollution, we can conclude that the researched areas are not polluted with this heavy metal. Following the analyzes carried out in the period 2012-2017 [27-31], in the four research sites (forestry, *agricultural, rural, urban approaches*), we found that the content of Pb in honey samples analyzed varies year function and is very close to the specificity of the site where the apiary was located (table 10). The presence of Pb was found in all honey samples analyzed in both years of research, ranging from a minimum of 0.008 mg/kg (the *forest* site) to a maximum of 0.311 mg/kg (urban proximity site).

At the same time, we note that none of the concentrations recorded in the honey collected in the mentioned localities exceeds the LMA established by European and International rules (0.20 mg/kg). In the year 2015, the average concentrations of Pb in honey samples at the *urban approach* site (0.162±0.021 mg/kg) and the *rural* site (0.138±0.010 mg/kg) were significantly higher compared to the site (0.017±0.002 mg/kg), respectively - by 0.145 and 0.121 mg/kg or 8.5% and 7.1 times (td = 6.90 - 7.50; $p < 0.001$). A much smaller difference compared to the *urban and rural* sites was found in Pb content in honey extracted from the *agricultural* site, being higher than the *forest* site by 0.006 mg/kg or 35.9% (td = 2.68; $p < 0.05$).

A similar situation was also found in the following year of study, where the concentration of Pb in honey from the rural site (0.133±0.013 mg/kg) was significantly higher than in the *forest* site (0.015±0.001 mg/kg) with 0.118 mg/kg or 8.8 times (td = 9.07, $p < 0.001$). The concentration of Pb in honey in the *agricultural* site had only a tendency

Region	Average concentration, M±m	Min.-max.
Year 2017		
Brusturi	0.134±0.010	0.115-0.153
Poiana	0.055±0.009	0.04 – 0.071
Tarzia	0.081±0.010	0.061- 0.095
LMA (UE,FAO/OMS)	0.20	

Table 9
Pb CONTENT IN HONEY (mg/kg)

Name of the experimental site	Average concentration, M±m	The difference from the blank site		td	p
		d	%		
Year 2015					
The "forest" site	0.017±0.002	-	-	-	-
The "agricultural" site	0.023±0.002	0.006	35.9	2.68	< 0.05
The "rural" site	0.138±0.010	0.121	711.7	7.50	< 0.001
The site "Urban approaches"	0.162±0.021	0.145	852.9	6.90	< 0.001
Year 2017					
The "forest" site	0.015±0.001	-	-	-	-
The "agricultural" site	0.019±0.002	0.004	26.6	1.78	< 0.1
The "rural" site	0.133±0.013	0.118	786.6	9.07	< 0.001
LMA, FAO/OMS**	max. 0.20	-	-	-	-

Table 10
Pb CONTENT IN HONEY (mg/kg)

Remark: * EU Standard (European Honey Directive of the European Honey Commission), Council Directive 2001/110/EC relating to honey [32];
** International Standard (Codex Alimentarius Standard of F.A.O./O.M.S Commission) [26].

to be higher than the background site - by 0.004 mg/kg or 26.6% (td = 1.78, p < 0.1).

Therefore, we can conclude from the research results that honey samples extracted from the affected sites, such as the *urban and rural* sites, which are permanently exposed to the diversity of anthropogenic activities, have a higher Pb concentration than in sites isolated, such as the *forest* site.

Also the aim of research consists in the analysis of the energy efficiency that is specific for the honey production in Neamt County, Romania. This is justified by the increase of inputs consumption in general and in the apiculture in specific, aiming the increasing of production [25]. Due to the low living level of the population of about 245 euro/pers (http://www.insse.ro), the management applied by them is not accordingly to the efficiency increase and consumption reduction, but is targeting short term objectives like the incomes and profit.

The objectives of the research are: determination of the main indicators related to the energy efficiency of the inputs use (energy use efficiency, energy productivity, specific energy and net energy); determination of the energy consumption structure (human labor, electricity, diesel fuel, drug, sugar and track, direct and indirect, renewable and non-renewable); identification of the inputs correlation and regression in proportion to the outputs.

These objectives are justified by the necessity to increase the efficiency of the energy inputs efficiency and the dynamic reduction of the natural environment degradation.

The main indicators of energy efficiency for the inputs use (table 1) illustrates an average energy input of 628 $MJ{\cdot}hive^{-1}$ and an average energy output of 235 $MJ{\cdot}hive^{-1}$. According to the apiaries size, it is noticed that the highest energy consumption have the apiaries over 151 have (1,074 $MJ{\cdot}hive^{-1}$), while the lowest energy consumption have the apiaries with less than 50 have (555 $MJ{\cdot}hive^{-1}$). The hives producing the highest energy quantity belong to the apiaries with 101-150 hives (449 $MJ{\cdot}hive^{-1}$). The average honey production is about 18.9 $kg{\cdot}hive^{-1}$ with variations from 16.1 $kg{\cdot}hive^{-1}$ (0-50) to 35.3 $kg{\cdot}hive^{-1}$ (101-150).

The net energy index shows that the specific loss of honey production amounts to 393.4 $MJ{\cdot}hive^{-1}$. The apiaries size and the management applied accordingly determines large variations. The apiaries over 151 hives lose with 76.0% more energy that the average, while the apiaries with less than 50 hives lose with 10.6% less energy than the average.

The average energy productivity is about 0.030 $kg{\cdot}MJ^{-1}$, the most productive apiaries being the apiaries with 101-150 hives (26.1% over average), while the less productive are the apiaries with less than 50 hives (3.5% under average). The energy use efficiency has maximum values at the apiaries with 101-150 haves (with 28.9% under average) and minimum values at the apiaries with more than 151 hives (2.2% under average). The apiaries with 101-150 hives manage the best the energy consumptions, having a minimum specific energy consumption (20.7% under average), while the apiaries with less than 50 hives have the highest consumption in order to obtain 1 kg honey (3.6% over average) (table 11).

Out of the total energy inputs, the fuel has the highest value reaching 329.5 $MJ{\cdot}hive^{-1}$ (table 12), representing 52.4% with variations from 31.2% (>151 hives) to 56.1% (0-50 hives). The largest part of this consumption is destined for the bee apiaries movements with the automobiles owned by the beekeepers. In addition comes the consumption with the transport carried out by third parties (track), with an average weight of 17.6% out the total inputs. Per total, the apiaries transport during movements needs about 70.0% of the apiaries energy consumption. An important input is represented by sugar (average of 24.3%) which at the apiaries with 101-150 hives has an average weight of 36.8% out of total inputs.

The determination of the indirect inputs illustrated a maximum consumption of 691 $MJ{\cdot}hive^{-1}$ at the apiaries with 51-100 have (table 13) representing 65.6% out of total inputs compared to the sample mean of 70.1%. The maximum direct inputs were identified at the apiaries with over 151 hives (439 $MJ{\cdot}hive^{-1}$).

The renewable inputs represent 5.3% out of the total inputs per sample with a maximum of 4.3% at the apiaries with 101-150 hives (table 13). The highest quantity of non-renewable energy was consumed by the hives belonging to the apiaries with more than 151 hives (1,038.8 $MJ \cdot hive^{-1}$)

The function estimated for the labour consumption (fig. 1), with the form 12.1 x - 170.6, shows that the threshold above which the labour will determine energy output is about 170.6 $MJ{\cdot}hive^{-1}$, but doesn't offer information about the maximum level up to which this determines an output increase.

Index	0-50 have	51-100 have	101-150 have	>151 have	Mean
Input energy (MJ·hive^{-1})	555	1054	933	1074	628
Output energy (MJ·hive^{-1})	203	398	449	382	235
Performance (MJ·hive^{-1})	16.1	34.0	35.3	32.0	18.9
Net energy (MJ·hive^{-1})	-351.8	-655.3	-483.3	-692.4	-393.4
Energy productivity (kg·MJ^{-1})	0.029	0.032	0.038	0.030	0.030
Energy use efficiency	0.37	0.38	0.48	0.36	0.37
Specific energy (MJ·kg^{-1})	34.5	31.0	26.4	33.6	33.3

Table 11
THE MEAN OF ENERGY INDICES

Index	0-50 have	51-100 have	101-150 have	>151 have	Mean
Inputs	554.8	1053.5	932.7	1074.5	628.3
Labor	32.0	44.6	40.2	35.7	33.6
Fuel	311.2	479.4	373.5	335.0	329.5
Electricity	1.0	3.0	6.1	9.3	1.6
Track	91.3	211.6	169.2	300.5	110.7
Drug	0.1	0.3	0.7	0.5	0.1
Sugar	119.3	314.6	343.1	393.5	152.9

Table 12
THE MEAN INPUTS (MJ·hive^{-1})

Index	0-50 have	51-100 have	101-150 have	>151 have	mean
Indirect input	402.4	691.0	542.7	635.5	440.2
Direct inputs	152.3	362.5	390.0	439.0	188.1
Renewable input	32.0	44.6	40.2	35.7	33.6
Nonrenewable input	522.8	1008.9	892.5	1038.8	594.8

Table 13
THE MEAN INPUTS

The fuel consumption, with a function estimated to have the form 0.3 x + 119.4 (fig. 2), is necessary to obtain an output with an energy value higher than 119.4 MJ·hive^{-1}. Without to consume fuel, the apiaries can obtain a honey production of 9.4 kg honey/hive.

Also, the function track inputs estimated to have the form 1.1 x + 112.5 (fig. 3) illustrates that, without to consume fuel, the apiaries can obtain 112.5 MJ·hive^{-1} (8.8 kg honey/hive).

The same situation is recorded also for the sugar consumption (fig. 4), for which was estimated a function with the form 0.8 x + 117.6, according to which the apiaries have enough nectar to produce 117.6 MJ·hive^{-1} (9.2 kg honey/hive).

By the other hand, a higher consumption of one or several factors - as labor, fuel, track and sugar – might supply information about the maximum level of efficiency that can be reached by using them.

For the inputs electricity and drugs, no satisfactory correlation had been determined (Pearson Correlation 0.436, respectively 0.479), and the regression wasn't statistically ensured.

The factors covariance by Stepwise method supplied the regression equation statistically ensured with the form -47.078 + 0.376 x_1 + 4.600 x_2 +0.134 x_3 + 183.981 x_4 where: sugar (x_1), labor (x_2), fuel (x_3), drug (x_4). This indicates the necessity of an input consumption with an energetic value of 47.1 MJ hive^{-1} over which the apiaries

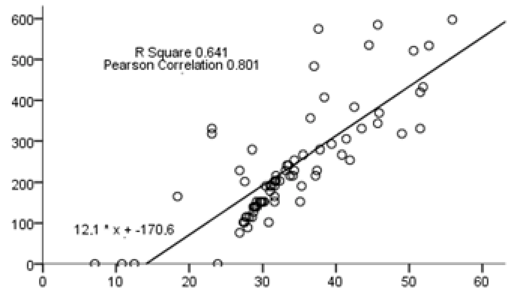

Fig.1. Polynomial regression trend lines of output energy (labour MJ·hive^{-1})

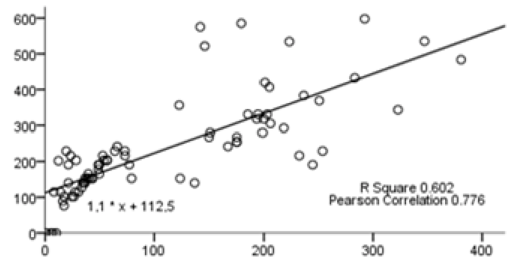

Fig.3. Polynomial regression trend lines of output energy (track MJ·have^{-1})

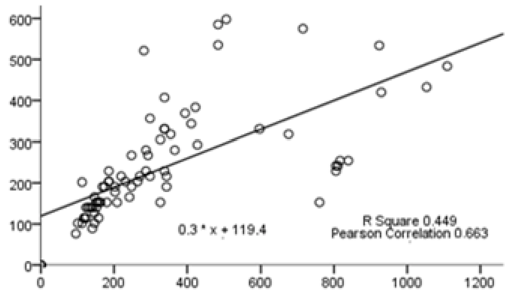

Fig.2. Polynomial regression trend lines of output energy (fuel MJ·hive^{-1})

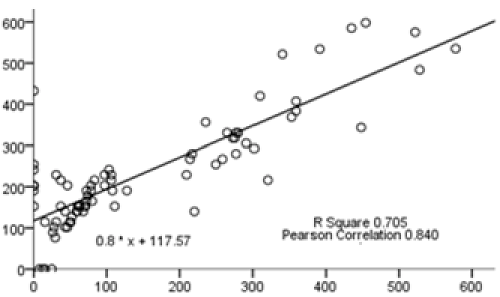

Fig.4. Polynomial regression trend lines of output energy (sugar MJ·hive^{-1})

Table 14
COEFFICIENTS DEPENDENT VARIABLE: OUTPUT (HONEY MJ·hive^{-1})

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
4	(Constant)	-47.078	23.396		-2.012	.048	-93.717	-0.439
	Sugar	0.376	0.052	0.411	7.223	.000	0.272	0.480
	Labor	4.600	0.881	0.305	5.223	.000	2.844	6.356
	Fuel	0.134	0.027	0.254	4.935	.000	.080	0.189
	Drug	183.981	38.234	0.226	4.812	.000	107.763	260.198

can obtain output (table 14). The average drugs coefficient has a high value because their specific consumption in energy units is very low (0.14 MJ·hive^{-1}).

Conclusions

Regarding the study on the monitoring of heavy metals residues in honey, we can draw the following conclusions:

The analysis revealed the presence of Zinc (0-1.48 ppm), Chromium (6.88-10.35 ppm), Nikel (0.664 - 7.64 ppm), Copper (10.73±55.52 ppm) and Iron (13.75±74.14 ppm) in the collected samples.

Contamination by Cadmium was found to be below detection level.

The presence of these toxic metals in bee honey is an evidence of micropolluting agents in the environment. Honey produced in different regions of Timis County do not completely lack heavy metals but they are at satisfactory level and good quality for human consumption:

-The total energy consumption specific for the honey production in Neamt County amounted 628 MJ·hive^{-1} and the energy production reached 235 MJ·hive^{-1}, determining an energy productivity of 0.030 kg·MJ^{-1} and an energy use efficiency of 0.37.

-The specific energy reached 33.3MJ/kg due to the inefficiency of apiaries movements and the inappropriate correlation between the apiaries size and the zonal melliferous potential.

-The highest inputs are those concerning the transport and fuel, representing 80.3% out of total inputs (45.9%, respectively, 34.4%).

-The indirect inputs reached 768.5 MJ hive^{-1} representing 80.3% out of total inputs under the conditions when the most important increase of the energy efficiency might be obtained through the increase of labor consumption and the additional treatments.

-The non-renewable energy represents 96.5% out of the consumed total energy, indicator that can be improved by executing with higher efficiency all agricultural works, as the bees swarming, nest organization for wintering, nest resizing in the active season and the families preparation for the production harvest.

The energy efficiency specific for the honey production in Neamt County registered low values due to the high inputs level, but mainly due to the low outputs level. The geo/climate conditions determine the obtaining of an average production of 18.9 kg·hive^{-1}, situation that determines some beekeepers to move to zones with high melliferous potential or to increase the number of hives. But, by one hand, in the most cases, the consumptions necessary for movements are higher than the obtained production. For this reason, the highest energy inputs are those concerning the fuel and track (768.5 MJ·hive^{-1}). The apiaries with les than 50 hives consume 25.0 MJ kg/honey, while those with 101-150 hives consume 15.4 MJ kg/honey, because the means of transport are inefficiently used. The beekeepers with small apiaries use their own means of transport that determine a high consumption km/tone. By the other hand, the increase of the bee families number isn't correlated with the zonal melliferous potential. Some zones can ensure a satisfactory productivity for 60-70 hives, while other zones are insufficient even for 30 hives. These data are valid when the hives honey consumption during winter is with 30-50% higher than in the hills and plan zones.

The linear regression illustrates that 1 MJ hive^{-1} of consumed sugar determines an output of 0.376 MJ·hive^{-1} and an energy efficiency increase with 0.006 compared to the sample mean.

A consumed fuel unit of MJ·hive^{-1} determines an output of 0.134 MJ·hive^{-1} and a decrease of energy efficiency with 0.236 compared to the sample mean.

By the other hand, a labor or drug consumption of 1 MJ hive^{-1} determines an output of 4.6 MJ·hive^{-1} respectively 183.981 MJ·hive^{-1} and an increase of energy efficiency with 4.23 MJ·hive^{-1} and respectively with 183.61 MJ·hive^{-1}.

The insufficient use of the labor factor to increase the honey production efficiency is justified by the apiaries size that doesn't ensure a substantial income for the beekeepers and, consequently, these are not perceived as a priority compared to other activities. Thus, a part of works are superficially accomplished or are neglected: the bees swarming nest organization for wintering, nest resizing in the active season and the families preparation for the production harvest etc.

The opportunity of drugs consumption increase may be partially seen with suspicion, but it is known the fact that the beekeepers from the researched area don't use treatments integrated schemes in order to control varroa disease and to fight against nosema disease. In generally, the treatments are insufficient and are carried out superficially.

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